

## Short Communication

## E17K substitution in AKT1 in prostate cancer

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**BACKGROUND:** The phosphatidylinositol 3-kinase (PI3K)–AKT pathway is activated in many cancers. Mutational hotspots in *AKT1* and in the regulatory and catalytic subunits of *PI3K* have been detected in multiple tumour types. In *AKT1*, the E17K substitution leads to a PI3K-independent activation of *AKT1*.

**METHODS:** A mutational profiling of *AKT1* and of the mutational hotspots in *PIK3CA* and *PIK3R1* was carried out in samples from primary and recurrent prostate tumours.

**RESULTS:** We show that, in prostate cancer, *AKT1*(E17K) had a prevalence of 1.4%. The mutation seemed to be associated with a favourable clinical course but it was not associated with a specific tumour growth pattern. Activating mutations in *PIK3CA* or *PIK3R1* were not found in prostate cancer.

**CONCLUSION:** The E17K substitution in *AKT1* is rare in prostate cancer. It seems associated with a favourable clinical outcome but not with a specific histology of the tumour.

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The phosphatidylinositol 3-kinase (PI3K)–v-akt murine thymoma viral oncogene homologue (AKT) signalling pathway is involved in cellular processes such as cell growth, proliferation, apoptosis, and cytoskeletal rearrangement. PI3K functions by catalysing the production of phosphorylated phosphoinositides (PtdIns). The PI3K phosphorylates PtdIns(4,5)P<sub>2</sub> (PIP<sub>2</sub>) into PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>), which binds to the pleckstrin homology domain of the downstream target, v-akt murine thymoma viral oncogene homologue 1 (AKT1). This results in a recruitment of AKT1 to the plasma membrane in which regulatory amino-acid residues serine 473 (Ser473) and threonine 308 (Thr308) are phosphorylated and activated (Vivanco and Sawyers, 2002).

The tumour-suppressor gene phosphatase and tensin homologue (*PTEN*) directly antagonises the PI3K–AKT pathway by converting PIP<sub>3</sub> back to PIP<sub>2</sub>. Absence of *PTEN* leads to increased phosphorylation of AKT (Cantley and Neel, 1999), thereby stimulating PI3K–AKT signalling. *PTEN* inactivation is frequent in various cancers, including prostate cancer (Li *et al*, 1997). It can occur by deletion (Cairns *et al*, 1997; Vlietstra *et al*, 1998; Verhagen *et al*, 2006), mutation (Suzuki *et al*, 1998; Vlietstra *et al*, 1998; Verhagen *et al*, 2006), or by decreased expression (Whang *et al*, 1998). Prostate-targeted *Pten* knockout mice develop prostate hyperplasia, intraepithelial neoplasia, and ultimately invasive cancer (Wang *et al*, 2003; Ma *et al*, 2005).

Activating mutations in *AKT1* or *PI3K* are other mechanisms that lead to stimulation of the PI3K–AKT pathway. PI3K is a heterodimer composed of a regulatory subunit (p85 $\alpha$ ), encoded by *PIK3R1*, and a catalytic subunit (p110 $\alpha$ ), encoded by *PIK3CA*. *PIK3CA* has frequently been reported as being mutated in various human cancers (Samuels *et al*, 2004; Levine *et al*, 2005).

*PIK3R1* mutations are less common, although recently it was shown that *PIK3R1* was mutated in up to 10% of glioblastomas (Cancer Genome Atlas Research Network, 2008; Parsons *et al*, 2008). Mutations in either gene lead to a disruption of the interaction between the regulatory and catalytic subunits, which enhances enzymatic activity (Huang *et al*, 2007), thereby activating the PI3K–AKT pathway. In prostate cancer, however, activating mutations in *PIK3CA* or *PIK3R1* have not been reported thus far (Majumder and Sellers, 2005; Ligresti *et al*, 2009).

Recently, a unique mutation in the pleckstrin homology domain of *AKT1* was identified in breast, ovarian, and colorectal cancer (Carpten *et al*, 2007). This G>A mutation results in a lysine substitution for glutamate at position 17 (E17K) and leads to a PI3K-independent activation of AKT1. The mutation was mutually exclusive with respect to mutations in *PI3K* and loss of *PTEN* protein expression. Others confirmed the mutation in breast and colorectal tumours (Bleeker *et al*, 2008) and incidental cases were identified in bladder, endometrial, lung, and skin cancer (Davies *et al*, 2008; Do *et al*, 2008; Malanga *et al*, 2008; Zilberman *et al*, 2009; Shoji *et al*, 2009). An analogous E17K substitution in AKT3 has also been found in melanomas (Davies *et al*, 2008). Previously, we reported on the E17K substitution in AKT1 in a ductal adenocarcinoma of the prostate in a patient who had a very long cancer-specific survival (Boormans *et al*, 2008).

In this study, we analysed the prevalence of AKT1(E17K) in a larger cohort of prostate cancer patients. We investigated whether the E17K substitution in AKT1 was associated with a specific growth pattern of prostate cancer and whether it corresponded with clinical outcome.

## MATERIALS AND METHODS

## AKT1

Genomic DNA was available from 184 freshly frozen clinical prostate cancer samples. A total of 85 samples were primary

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prostate tumours obtained by radical prostatectomy, 88 samples were locally advanced or recurrent tumours obtained by transurethral resection of the prostate, and 11 samples were hormone-naïve prostate cancer lymph node metastases obtained by pelvic lymph node dissection. An additional 30 formalin-fixed, paraffin-embedded primary prostate tumours were selected, including 18 ductal adenocarcinomas of the prostate and 12 primary prostate tumours with the following characteristics: pathological T stage  $\geq$  pT3a, prostate-specific antigen level  $\geq 4.0 \text{ ng dl}^{-1}$ , any Gleason score, and a cancer-specific survival of  $>13$  years. From the cancerous regions of the paraffin-embedded samples, 1-mm core biopsy samples were taken (Beecher Instruments, Silver Spring, MD, USA). In mutated samples, a core biopsy sample was taken from adjacent benign prostatic tissue to test whether the mutation was truly somatic. Genomic DNA was isolated using the Puregene DNA isolation kit (BIOzym, Landgraaf, the Netherlands), according to the manufacturer's instructions.

PCR analysis was carried out to yield a 198-bp genomic fragment of *AKT1* (see Supplementary Table 1 for primer sequences). PCR products were identified by 2% agarose gel electrophoresis and ethidium bromide staining. After ExoSapI (USB, Staufien, Germany) treatment, the PCR fragments were sequenced with the reverse *AKT1* primer. Sequence reaction products were analysed on the ABI 3700 automated DNA sequencer (Applied Biosystems, Carlsbad, CA, USA). The freshly frozen samples containing the E17K substitution in *AKT1* were also analysed for *PTEN* mutations and for mutations in the mutational hotspots of exons 9 and 20 of *PIK3CA* (see Supplementary Table 1 for primer sequences of *PIK3CA* and *PTEN*).

### *PIK3CA* and *PIK3R1*

Mutational analysis of *PIK3CA* and *PIK3R1* was also carried out on a subset of 63 freshly frozen tissue samples: 61 transurethral resection of the prostate samples and two lymph node metastases. The exons known to contain mutational hotspots were sequenced, that is, exons 9 and 20 for *PIK3CA* and exons 14 and 15 for *PIK3R1* (see Supplementary Table 1 for primer sequences).

## RESULTS AND DISCUSSION

The PI3K–AKT signalling pathway is a central factor in various cellular processes that are involved in carcinogenesis. The E17K substitution in *AKT1* is an important mechanism that leads to a PI3K-independent activation of *AKT1* (Carpten *et al*, 2007). Rare E17K substitution in *AKT3* has also been described (Davies *et al*,

2008). In this study, we focussed on the more common *AKT1*(E17K) substitution in prostate cancer. Previously, we identified *AKT1*(E17K) in a pure ductal adenocarcinoma of the prostate in a patient who had a long survival (Boormans *et al*, 2008). Here, we analysed an additional 18 ductal prostate cancer samples; however, none of these samples contained the E17K substitution in *AKT1*. Therefore, we concluded that an association between *AKT1*(E17K) and a specific ductal growth pattern of prostate cancer is unlikely. This is in contrast to *AKT1*(E17K) in breast and lung cancer. In breast cancer, the mutation was unique for lobular and ductal histotypes (Bleeker *et al*, 2008), whereas in lung tumours *AKT1*(E17K) was only seen in squamous cell carcinomas (Bleeker *et al*, 2008; Do *et al*, 2008; Malanga *et al*, 2008).

Next, we randomly extended our search for *AKT1*(E17K) in 184 freshly frozen clinical prostate cancer samples. One extra patient harbouring the mutation was identified. The sample was a primary prostate tumour obtained by radical prostatectomy. The tumour was a moderately differentiated adenocarcinoma (Gleason score  $3+3=6$ ) with bladder neck involvement (pathological T stage: pT4a) and positive surgical margins. Moreover, occult pelvic lymph node metastases were present at the time of radical prostatectomy. Despite these prognostic unfavourable characteristics, the patient is still alive at the end of follow-up (survival  $\sim 17$  years). These findings were in agreement with the clinical course of the patient we described in our previous report (Boormans *et al*, 2008). That patient was diagnosed with a poorly differentiated adenocarcinoma (Gleason score  $4+4=8$ ), but he also had a long survival ( $>18$  years) and he did not die from prostate cancer (see Table 1a for the clinical and histopathological characteristics of the patients).

To investigate whether *AKT1*(E17K) in prostate cancer was associated with a favourable clinical outcome, as suggested by the first two patients harbouring the *AKT1* mutation, we selected an additional 12 paraffin-embedded primary prostate tumours. All patients had unfavourable clinicopathological characteristics; nevertheless, they had a survival of  $>13$  years (see Materials and Methods section for selection criteria). In these additional 12 prostate tumours, we identified one extra patient having the E17K substitution in *AKT1*. Pathology showed a moderately differentiated adenocarcinoma (Gleason score  $3+3=6$ ) with extracapsular extension (pathological T stage: pT3a) and positive surgical margins. Almost 18 years after the radical prostatectomy, the patient died from causes other than prostate cancer.

In a recent series from our institution on patients with clinical T3 prostate tumours who were treated by radical prostatectomy and pelvic lymphadenectomy, cancer-specific and overall survival

**Table 1a** Clinical and histopathological characteristics of three prostate cancer patients harbouring the E17K substitution in *AKT1*

Patient	Age at diagnosis (years)	Initial PSA (ng ml <sup>-1</sup> )	Primary treatment	Secondary treatment	Tertiary treatment	cT-stage	pT-stage	Gleason score
I	74	8.8	WaWa	TURP	TURP+ET	cT2b	NA	4+4=8
II	72	13.0	RP and PLND	None	None	cT2b	pT4a	3+3=6
III	62	5.6	RP and PLND	None	None	cT3x	pT3a	3+3=6

  

Patient	Surgical margins	N+	Tissue	Histotype	Death	OS (years)	PCa death	CSS (years)
I	NA	NA	TURP freshly frozen	Ductal adenocarcinoma	Yes	18.4	No	$\geq 18.4$
II	Positive	Yes	RP freshly frozen	Acinar adenocarcinoma	No	$\geq 17.0$	No	$\geq 17$
III	Positive	No	RP PEFF	Acinar Adenocarcinoma	Yes	16.8	No	$\geq 16.8$

Abbreviations: CSS = cancer-specific survival; cT-stage = clinical T stage; ET = endocrine therapy; NA = not applicable; OS = overall survival; PCa death = prostate cancer death; PLND = pelvic lymph node dissection; PEFF = paraffin-embedded, formalin fixed; PSA = prostate-specific antigen; pT-stage = pathological T stage; RP = radical prostatectomy; TURP = transurethral resection of the prostate; WaWa = watchful waiting.

**Table 1b** Genetic characteristics of three prostate cancer patients harbouring the E17K substitution in AKT1

Patient	Sequence of PTEN exons	Sequence of PIK3CA exons 9 and 20	Sequence of PIK3RI exons 14 and 15
I	Wild type	Wild type	Wild type
II	Wild type	Wild type	Unknown
III	Unknown	Unknown	Unknown

Abbreviation: PTEN = phosphatase and tensin homologue.

after 15 years was 66 and 37%, respectively (Hsu *et al*, 2009). In a recent review, it was hypothesised that the presence of AKT1(E17K) is associated with a less-aggressive form of cancer (Brugge *et al*, 2007). The findings of our present series could be in agreement with such a hypothesis: all three patients harbouring the E17K substitution in AKT1 had a very long survival despite aggressive clinicopathological characteristics. Obviously, the findings of this study do not prove an association with better outcome because of the low prevalence of AKT1(E17K) in prostate cancer.

## REFERENCES

Bleeker FE, Felicioni L, Buttitta F, Lamba S, Cardone L, Rodolfo M, Scarpa A, Leenstra S, Frattini M, Barbareschi M, Del Grammastro M, Sciarrotta MG, Zanon C, Marchetti A, Bardelli A (2008) AKT1 (E17K) in human solid tumours. *Oncogene* 27: 5648–5650

Boormans JL, Hermans KG, van Leenders GJLH, Trapman J, Verhagen PCMS (2008) An activating mutation in AKT1 in human prostate cancer. *Int J Cancer* 123: 2725–2726

Brugge J, Hung MC, Mills GB (2007) A new mutational activation in the PI3K pathway. *Cancer Cell* 12: 104–107

Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455: 1061–1068

Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D (1997) Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res* 57: 4997–5000

Cantley LC, Neel BG (1999) New insights into tumor progression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci USA* 96: 4240–4245

Carpten JD, Faber AL, Horn C, Donoho GP, Briggs S, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savaga S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellog G, Touchman J, Patel K, Mousset S, Brittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE (2007) A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 448: 439–444

Davies MA, Stemke-Hale K, Tellez C, Calderone TL, Deng W, Prieto VG, Lazar AFJ, Gershenwald JE, Mills GB (2008) A novel AKT3 mutation in melanoma tumours and cell lines. *Br J Cancer* 99: 1265–1268

Do H, Solomon B, Mitchell PL, Fox SB, Dobrovic A (2008) Detection of the transforming AKT1 mutation E17K in non-small cell lung cancer by high resolution melting. *BMC Res Notes* 1: 14

Hsu CY, Wildhagen MF, van Poppel H, Bangma CH (2009) Prognostic factors for and outcomes of locally advanced prostate cancer after radical prostatectomy. *BJU Int*; e-pub ahead of print 12 November 2009

Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, Vogelstein B, Gabeli SB, Amzel LM (2007) The structure of a human p110 $\alpha$ /p85 $\alpha$  complex elucidates the effects of oncogenic PI3K $\alpha$  mutations. *Science* 318: 1744–1748

Levine DA, Bogomolny F, Yee CJ, Lash A, Barakat RR, Borgen PI, Boyd J (2005) Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res* 11: 2875–2878

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Milaresis C, Rodgers L, McCombie R, Bigner SH, Giovannella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein

The mutational profiling of the mutational hotspots of PIK3CA and PIK3RI in a subset of 63 prostate cancer patients did not reveal any mutated sample, which is consistent with earlier findings (Ligresti *et al*, 2009).

In conclusion, we have identified three patients harbouring E17K substitution in AKT1 in prostate cancer, rendering the reported frequency for AKT1(E17K) in prostate cancer at 1.4% (3 out of 214 patients examined). The mutation showed to be truly somatic, as the sequence of AKT1 derived from benign prostatic tissue of patients carrying the mutation was of wild type (data not shown). AKT1(E17K) seemed to be associated with a favourable clinical course, but no correlation with a ductal growth pattern of prostate cancer was found. Furthermore, the E17K substitution in AKT1 was mutually exclusive with respect to mutations in PTEN and PIK3CA, as in the freshly frozen samples, mutational profiling of all nine PTEN exons and of the mutational hotspots of PIK3CA (exons 9 and 20) showed no mutations (Table 1b). The possibility of loss of PTEN function by loss of expression was not assessed in this study.

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tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 275: 1943–1947

Ligresti G, Militello L, Steelman LS, Cavallaro A, Basile F, Nicoletti F, Stivala F, McCubrey JA, Libra M (2009) PIK3CA mutations in human solid tumours. *Cell Cycle* 8: 1352–1358

Ma X, Ziel-van der Made A, Autar B, van der Korput A, Vermeij M, van Duijn P, Cleutjens KB, de Krijger R, Krimpenfort P, Berns A, van der Kwast TH, Trapman J (2005) Targeted biallelic inactivation of Pten in the mouse prostate leads to prostate cancer accompanied by increased epithelial cell proliferation but not by reduced apoptosis. *Cancer Res* 65: 5730–5739

Majumder PK, Sellers WR (2005) Akt-regulated pathways in prostate cancer. *Oncogene* 24: 7465–7474

Malanga D, Scrima M, De Marco C, Fabiani F, De Rosa N, De Gisi S, Malara N, Savino R, Rocco G, Chiappetta G, Franco R, Tirino V, Pirozzi G, Viglietto G (2008) Activating E17K mutation in the gene encoding the protein kinase AKT1 in a subset of squamous cell carcinoma of the lung. *Cell Cycle* 7: 665–669

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz Jr LA, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. *Science* 321: 1807–1812

Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JKV, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) High frequency of mutations of the PIK3CA gene in human cancers. *Science* 304: 554

Shoji K, Oda K, Nakagawa S, Hosakawa S, Nagae G, Uehara Y, Sone K, Miyamoto Y, Hiraike H, Hiraike-Wada O, Nei T, Kawana K, Aburatani H, Yano T, Taketani Y (2009) The oncogenic mutation in the pleckstrin homology domain of AKT1 in endometrial carcinomas. *Br J Cancer* 101: 145–148

Suzuki H, Freije D, Nusskern DR, Okami K, Cairns P, Sidransky D, Isaacs WB, Bova GS (1998) Interfocal heterogeneity of PTEN/MMAC1 gene alterations in multiple metastatic prostate cancer tissues. *Cancer Res* 58: 204–209

Verhagen PC, van Duijn PW, Hermans KG, Looijenga LH, van Gurp RJ, Stoop H, van der Kwast TH, Trapman J (2006) The PTEN gene in locally progressive prostate cancer is preferentially inactivated by bi-allelic gene deletion. *J Pathol* 208: 699–707

Vivanco I, Sawyers CL (2002) The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nature Rev Cancer* 2: 489–501

- Vlietstra RJ, van Alewijk DC, Hermans KG, van Steenbrugge GJ, Trapman J (1998) Frequent inactivation of PTEN in prostate cancer cell lines and xenografts. *Cancer Res* **58**: 2720–2723
- Wang S, Gao L, Lei Q, Rozengurt N, Pritchard C, Jiao J, Thomas GV, Li G, Roy-Burman P, Nelson PS, Liu X, Wu H (2003) Prostate-specific deletion of the murine Pten tumour suppressor gene leads to metastatic prostate cancer. *Cancer Cell* **4**: 209–221
- Whang YE, Wu X, Suzuki H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL (1998) Inactivation of the tumour suppressor PTEN/MMAC1 in advanced prostate cancer through loss of expression. *Proc Natl Acad Sci USA* **95**: 5246–5250
- Zilberman DE, Cohen Y, Amariglio N, Fridman E, Ramon J, Rechavi G (2009) AKT1 E17K pleckstrin homology domain in urothelial carcinoma. *Cancer Genet Cytogenet* **191**: 34–37